

Effects of fasting on IGF-I, IGF-II, and IGF-binding protein mRNA concentrations in channel catfish (*Ictalurus punctatus*)

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Abstract

The effects of fasting on insulin-like growth factor (IGF)-I, IGF-II, and IGF-binding protein (IGFBPs) mRNA in channel catfish were examined. Fed control fish (Fed) were compared to fish that had been fasted for 30 d followed by 15 d of additional feeding (Restricted). Sequence alignment and similarity to orthologous proteins in other vertebrates provided structural evidence that the 3 catfish sequences identified in the present research were IGFBP-1, -2, and -3. Prolonged fasting (30 d) reduced body weight approximately 60% ($P < 0.001$) and decreased IGF-I mRNA in the liver and muscle ($P < 0.01$). Fifteen days of re-feeding restored concentrations of hepatic and muscle IGF-I mRNA. Liver IGF-II mRNA was not affected by fasting but was increased 2.2-fold after 15 d of re-feeding ($P < 0.05$). Abundance of muscle IGF-II mRNA was similar between the fed control group and the restricted group throughout the experimental period. Fasting also increased liver IGFBP-1 mRNA ($P < 0.05$) and decreased IGFBP-3 mRNA ($P < 0.01$), whereas abundance of IGFBP-2 mRNA was not significantly affected. Interestingly, re-feeding for 15 d did not restore concentrations of IGFBP-1 and IGFBP-3 mRNA relative to fed control concentrations. The IGF results suggest that IGF-I and IGF-II are differently regulated by nutritional status and probably have a differential effect in promoting muscle growth during recovery from fasting. Similar to mammals, IGFBP-1 mRNA in catfish is increased during catabolism, whereas IGFBP-3 mRNA is decreased during inhibited somatic growth. The IGFBP results provide additional evidence of the conserved nature of the IGF-IGFBP-growth axis in catfish.

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Keywords: Fasting; IGF-I; IGF-II; IGF-binding proteins; Catfish

1. Introduction

The growth hormone-insulin-like growth factor (GH-IGF) axis has been implicated in the regulation of somatic growth and nutrient metabolism in both mammals and teleost fishes [1,2]. In fish and mammals, GH is a potent regulator of hepatic IGF-I expression [3,4], and several authors have reported that the IGF-II gene is

also regulated by GH, as administration of exogenous GH increases concentrations of IGF-II mRNA [5–8]. The activity of IGFs is regulated not only by GH and other endocrine modulators that enhance or suppress local and systemic IGF concentrations, but also by the presence of IGF binding proteins (IGFBPs). These binding proteins are crucial for modulating the half-lives of IGFs and coordinating and transporting IGFs in circulation [9–14]. In mammals, the IGFBPs include a family of 6 proteins (IGFBP-1 to -6) that bind to IGFs with high affinity and specificity [15]. Insulin-like growth factor binding proteins have been identified in several teleost species [16–22], including channel catfish

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[23–25]. Three teleost IGFBPs, ranging in size from 24 to 50 kDa, are commonly reported. The IGFBP family has recently expanded to include the IGFBP-related proteins (IGFBP-rPs), which may also play a role in regulating IGF activity [26]. The IGFs evoke their biological responses through their respective receptors on target tissues, resulting in growth promotion.

It is widely accepted that fasting or reduced feeding decreases concentrations of IGF-I in channel catfish (*Ictalurus punctatus*) and other teleosts [27–33], whereas the effects of nutritional status on IGF-II are less defined. Handling stress, confinement stress, and starvation result in decreased plasma concentrations of IGF-II in Atlantic salmon (*Salmo Salar*) and rainbow trout (*Oncorhynchus mykiss*) [34–35]. In channel catfish, IGF-II mRNA is up-regulated after GH administration and is greater in fast-growing compared to slow-growing families [8,36,37]. However, the effect of reduced feeding or starvation on IGF-II mRNA in channel catfish is not known.

Research with channel catfish has shown that nutritional status is a key regulator of IGFBP-1 and IGF-I in channel catfish. There is no information on how nutritional status governs IGFBP-2, IGFBP-3, or IGF-II mRNA concentrations in channel catfish, and the primary amino acid sequences of catfish IGFBPs are not known. The objectives of this study were to examine the effects of fasting on IGF-I, IGF-II, IGFBP-1, IGFBP-2, and IGFBP-3 mRNA in channel catfish, the primary cultured species in the southeastern United States.

2. Materials and methods

2.1. Research animals

Fish used in this study were a channel catfish strain (NWAC103) maintained by the National Warmwater Aquaculture Center (NWAC) and housed at the USDA-ARS Catfish Genetics Research Unit aquaculture facility in Stoneville, MS. Prior to experimentation, approximately 50 fish from each of 3 different families were placed into a 120-L holding tank. The following day, 100 fish (mean initial size 72.4 ± 2.1 g) were randomly assigned to ten 76-L tanks (10 fish/tank) and allowed to acclimate for 12 days. The fish were fed once per day to apparent satiation using a commercial 36% crude protein floating catfish diet (Farmland Industries, Inc., Kansas City, MO) and reared in 26.7 ± 0.2 °C flow-through well water under a 14:10 L:D photoperiod. Water quality (pH approximately 8.5; dissolved oxygen concentrations > 5.0 mg/L) and flow rates (1.0 L/min) were similar between tanks.

The fish were randomly separated into 2 treatment groups with 5 replicates each. One group served as fed controls (Fed) (commercial catfish diet, fed daily to apparent satiation), whereas the other group of fish was subjected to a 30-d fast followed by 15 additional days of refeeding (Restricted). On day 0 of the experiment, all fish were anesthetized with 0.1 g/L of tricainemethane sulfonate (MS-222; Western Chemical Inc., Ferndale, WA) and weighed. Five fish were sampled from the 120-L holding tanks and served as time 0 controls. On days 30 and 45, all fish were weighed and 15 fish per treatment (3 fish/tank) were euthanized with an overdose of MS-222 (0.3 g/L) for sample collection. Approximately 100 mg of liver and muscle (transverse slice of fast muscle located beneath the dorsal fin) were removed for RNA extraction. No mortalities were observed throughout the study. Studies were conducted in accordance with the principles and procedures approved by the Institutional Animal Care and Use Committee, United States Department of Agriculture/Agriculture Research Service Catfish Genetics Research Unit.

2.2. Sample preparation and RNA isolation

After collection, samples were immediately placed in 1 mL TRI-Reagent (Molecular Research Center, Inc., Cincinnati, OH), flash-frozen in liquid nitrogen, and stored at -80 °C until RNA isolation. Total RNA was isolated, quantified by measuring the absorbance at 260 nm using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Rockland, DE), and the integrity of the RNA was verified by visualization of the 18S and 28S ribosomal bands stained with ethidium bromide after electrophoresis on 2.0% agarose gels.

Extracted RNA was treated with DNase I to remove co-extracted DNA using a TURBO DNA-free™ kit (Ambion, Austin, TX). An aliquot of the extracted RNA (15 µL) was treated at 37 °C for 30 min with 0.1 volume of buffer (1.5 µL) and 2 U (1 µL) of DNase I. The enzyme was inactivated with 2 µL of inactivation reagent at room temperature for 2 min. Samples were centrifuged at $10\,000 \times g$ for 1 min, and the supernatant (16 µL) was transferred to a clean RNase-free microcentrifuge tube and stored at -80 °C before the reverse transcriptase step.

2.3. Identification and quantitation of catfish IGFBP mRNAs

Partial sequences for channel catfish IGFBP-1, -2, and -3 mRNAs were identified by sequence similarity with zebra fish (*Danio rerio*) IGFBPs using BlastX searches of catfish expressed sequence tags (ESTs). The full

Table 1

Nucleotide sequences of the PCR primers and probes used to assay gene expression by real-time quantitative PCR.

Gene	Oligonucleotide ^a	Sequence	Amplicon length
IGFBP-1	Sense	CAAGCTGTGTGCACTGAGATC	125 bp
	Antisense	ATCGTTGAGGCGGTTTCAGC	
	Probe	CAACTCGGGCTGATCTGGCGC	
IGFBP-2	Sense	GATGATGAACCGTGTGGATG	138 bp
	Antisense	TCATACAGGTTCTCCAGGTG	
	Probe	CTCGACCCAGAATGAGTCAATGTCAAC	
IGFBP-3	Sense	CAGAGCCACGGACAGAAAAG	122 bp
	Antisense	CAGACGCACGGACAGAAA	
	Probe	TCACCAGCTACCAAGAAGATGTC	

Abbreviation: PCR, polymerase chain reaction.

^a All probes were dual-labeled with 5'-FAM and 3'-BHQ-1 quencher.

coding sequences of IGFBP-1 and IGFBP-3 were determined by directed sequencing of BAC clone GY015J09 that contained both genes, but no genomic clone containing IGFBP2 could be identified in this library. Sequence similarity with other vertebrate IGFBPs was determined using pairwise ClustalW alignment. Genomic sequence verified intron-exon junctions in IGFBP-1 and IGFBP-3 were conserved with other vertebrates, thus IGFBP-2 intron-exon junctions were assumed to be conserved. For each gene, primers for quantitative real-time polymerase chain reaction (PCR) were designed in adjacent exons, and a dual-labeled probe was designed to span both exons to minimize detection of contaminating genomic DNA (Table 1).

Quantitative real-time PCR was performed using the iCycler iQ real-time PCR detection system (BioRad) to quantify IGF-I, IGF-II, and 18S rRNA, as previously described (Peterson et al., 2004). Amplification products specific to IGFBP were cloned into the pCR 4-TOPO vector (Invitrogen, Carlsbad, CA), and the identity of the cloned inserts was confirmed by DNA sequencing. The plasmid containing the correct insert for the respective gene was used as the PCR standard after DNA sequencing. One microgram of total RNA from each tissue was reverse-transcribed using the iScript cDNA Synthesis Kit (BioRad, Hercules, CA) according to the protocol provided by the manufacturer. Each amplification reaction mixture (12.5 μ L) contained 400 ng of cDNA; 1X iQ Supermix (Bio-Rad), 5.0 μ M dual-labeled probe, and 10 μ M (IGFBP-1, -2, and -3) of each primer. The real-time PCR protocol for IGFBP-1, -2, and -3 was 3 min at 95 °C; 45 cycles of 95 °C for 15 s, and 60 °C for 1 min. All amplifications were performed in triplicate. The standard curve showed a linear relationship between cycle threshold values and the logarithm of input gene copy number. We used 18S ribosomal RNA as an internal control to normalize respective genes because

other housekeeping genes changed with respect to treatment.

2.4. Statistical analysis

The statistical analyses were conducted as a 1-way analysis (ANOVA), with separate ANOVAs for each sampling interval (days 30 and 45) using Statistical Analysis System version 9.1 software (SAS Institute, Inc., Cary, NC) followed by a Duncan multiple-range test. Normalized gene expression data passed Levene's test for homogeneity of variance. Tissue gene expression concentrations and weight gain were subjected to ANOVA, with treatment as a fixed effect and tank within treatment as a random effect. Tank served as the experimental unit for each variable measured. Differences between treatments were considered significantly different at $P < 0.05$.

3. Results

The full deduced amino acid sequence was determined for catfish IGFBP-1 and IGFBP-3, and partial sequence was determined for IGFBP-2, through molecular cloning and sequencing of cDNA and genomic DNA. The 3 proteins shared <37% sequence identity (Table 2a) but contained localized regions of conserved sequence, including 12 conserved cysteine residues (Fig. 1). When compared with other teleosts, chickens, and humans, each IGFBP was most similar to the orthologous IGFBP in other species, for example, catfish IGFBP-1 was most similar to IGFBP-1 compared to -2 or -3 in other species (Tables 2a and 2b). Catfish IGFBP-1 was only 42.3% and 40.9% similar to IGFBP-1 in chickens and humans, respectively, but ranged from 52.9%–70.4% similar to teleost IGFBP-1 sequences. Catfish IGFBP-2 was 52.2%–61.2% similar to teleost, 50.0%

Table 2a

Percentage amino acid identity between insulin-like growth factor binding proteins (IGFBPs) of channel catfish and other teleosts.

Gene	Species	Accession	IGFBP1	IGFBP2	IGFBP3
IGFBP-1	<i>I. punctatus</i>	FJ668941	100	35.3	36.4
IGFBP-2	<i>I. punctatus</i>	FJ668942	35.3	100	35.8
IGFBP-3	<i>I. punctatus</i>	FJ668940	36.4	35.8	100
IGFBP-1a	<i>D. rerio</i>	NM.173283	70.4	34.3	35.6
IGFBP-1b	<i>D. rerio</i>	NM.001098257	52.9	25.5	34.5
IGFBP-2	<i>D. rerio</i>	NP_571533	33.2	54.9	34.5
IGFBP-2b	<i>D. rerio</i>	NP_001119936.1	32.9	61.2	34.5
IGFBP-3	<i>D. rerio</i>	NP_991314.2	32.5	26.8	56.4
IGFBP-5	<i>D. rerio</i>	NM.001126463	32.3	32.5	41.8
IGFBP-6like	<i>D. rerio</i>	XM.001922550	31.6	22.5	29.4
IGFBP-6like	<i>D. rerio</i>	XM.688133	32.8	23.5	29.0
IGFBP-1	<i>O. mykiss</i>	NM.001124561	57.0	26.9	30.3
IGFBP-2	<i>O. mykiss</i>	NM.001124649	35.0	56.5	35.6
IGFBP-3	<i>O. mykiss</i>	NM.001124557	31.9	54.8	29.7
IGFBP-4	<i>O. mykiss</i>	DQ146967	35.8	19.9	14.9
IGFBP-5	<i>O. mykiss</i>	NM.001124652	29.1	30.1	41.3
IGFBP-6	<i>O. mykiss</i>	DQ190459	31.2	23.5	32.3
IGFBP-rP1	<i>O. mykiss</i>	DQ146965	18.1	20.6	21.2
IGFBP-1	<i>S. salar</i>	NP_001117096.1	58.0	26.9	31.5
IGFBP-2	<i>S. salar</i>	NP_001117097.1	34.0	56.0	36.3
IGFBP-3	<i>S. salar</i>	NP_001117120.1	31.9	52.9	29.7
IGFBP-1	<i>S. quinquerediata</i>	ACD10797.1	56.9	26.4	35.8
IGFBP-2	<i>S. quinquerediata</i>	ACD11355.1	32.3	53.9	32.7
IGFBP-3	<i>S. quinquerediata</i>	ACD11356.1	30.4	29.7	56.4
IGFBP-1	<i>O. tshawytscha</i>	AAV83995.1	57.4	26.9	30.3
IGFBP-1	<i>G. mirabilis</i>	AAG13329.1	59.1	17.2	13.3
IGFBP-2	<i>D. labrax</i>	ACB15195.1	34.0	56.2	32.2
IGFBP-2	<i>S. aurata</i>	AAL57278.1	35.5	52.2	32.4
IGFBP-3	<i>O. mossambicus</i>	AAK97641.1	36.7	30.2	42.6

Note: Sequence comparisons were performed using pairwise ClustalW alignments. Identities greater than 50% are denoted in bold. Species and common names: *D. rerio* – zebra fish, *D. labrax* – European seabass, *G. mirabilis* – mudsucker, *O. mossambicus* – tilapia, *O. mykiss* – rainbow trout, *O. tshawytscha* – Chinook salmon, *S. aurata* – gilthead seabream, *S. quinquerediata* – Japanese amberjack, *S. salar* – Atlantic salmon, *T. nigroviridis* – spotted green pufferfish, *T. rubripes* – fugu.

similar to chicken, and 51.2% similar to human IGFBP-2 sequences. Catfish IGFBP-3 was 29.7%–56.4% similar to teleost, 52.0% similar to chicken, and 47.5% similar to human IGFBP-3 sequences.

Most instances of vertebrate sequence conservation were found in the N- and C-domains of these proteins. For example, catfish IGFBP-1 was 46.7% and 49.6% similar within the N-domain, 18.9% and 18.0% similar

Table 2b

Percentage amino acid identity between insulin-like growth factor binding proteins of channel catfish, chickens (*G. gallus*), and humans (*H. sapiens*).

Gene	Species	Accession	IGFBP1	IGFBP2	IGFBP3
IGFBP-1	<i>G. gallus</i>	NP.001001294.1	42.3	29.8	36.2
IGFBP-2	<i>G. gallus</i>	NP.990690.1	36.9	50.0	40.6
IGFBP-3	<i>G. gallus</i>	NP.001094504.1	30.1	31.1	52.0
IGFBP-4	<i>G. gallus</i>	NP.989684.1	38.2	37.1	35.7
IGFBP-5	<i>G. gallus</i>	XP.422069.2	27.8	15.9	14.0
IGFBP-1	<i>H. sapiens</i>	AAH57806.1	40.9	30.0	32.4
IGFBP-2	<i>H. sapiens</i>	AAA36048.1	36.6	51.2	34.8
IGFBP-3	<i>H. sapiens</i>	ABI63364.1	35.6	33.0	47.5
IGFBP-4	<i>H. sapiens</i>	AAV38694.1	38.8	41.3	37.3
IGFBP-5	<i>H. sapiens</i>	NP.000590.1	34.4	31.7	42.6

Note: Sequence comparisons were performed using pairwise ClustalW alignments. Identities greater than 50% are denoted in bold.

IGFBP-1	MSAVLLKLFHAVVLGAVLLAPQLRASPLLEPIRCAQCTAERVAECPVDAGCA	53
IGFBP-2	DLELGPSTAEQQAACPKPPSPCT	24
IGFBP-3	MKPLFRSVRLGSMAPRALWLTALFAALARTVHAAGPVVRCEPCDADALGQCKPLPRDCA	60
IGFBP-1	EVLREPGCGCCCLACAIKRGDS [•] CGIYTAPCGSGLRCLPTPGELRPLHALTRGQAVCTEIGE	113
IGFBP-2	ELVREPGCGCCPVCAIQLGEP [•] CGVYTARCGSGLRCS [•] PSEGSEMPLDDLKGLGSCQVRTE	84
IGFBP-3	ERVREPGCGCCLTCALSEGQAC [•] CGVYTGRCGAGLRCL [•] QHRPGESKPLQALLEGRGVCSRATD	120
IGFBP-1	DVQIRTQPAPDQPELEGDRSEASTGPETASTIVVSGHIKPFDPWLLSGAQESMSKSVNT	173
IGFBP-2	NTDT-----HDERDLES [•] DYGELYATRPPI [•] LRKPKVDANYWHMRETMPKHHQNEQKARMMN	139
IGFBP-3	-----RKL-	123
IGFBP-1	YRR--KLVEQGP---CHVELQRALEKIARS---QKLE [•] EKLT [•] KFYLPNC [•] DKQGLYKSKQ	224
IGFBP-2	RVDEPKIPRPRMSQ-CQQQLDKVLEEISKMTFHDNKGHIENLYELTFPNCDSNGQYNVQK	198
IGFBP-3	RVG--KRPNGISPLLCLFVSISLSFFLSFSPDVLFA-----	157
IGFBP-1	CESTLDGQ [•] RQ [•] GK [•] CWC [•] VT [•] SWNGKKIVGSSSELPTDAECPQEFNH	265
IGFBP-2	CHMSTHGQ [•] RGE...	209
IGFBP-3	-----CTGSYATFPIHVSSLASLNK [•] FVLISEH	184

Fig. 1. Amino acid sequence alignment of insulin-like growth factor binding protein (IGFBP)-1, IGFBP-3, and partial sequence of IGFBP-2 from channel catfish, *Ictalurus punctatus*. Regions of high sequence similarity are denoted within boxes. Alignment at the COOH-terminus was optimized to estimate conservation of cysteine (●) residues.

in the L-domain, and 52.4% and 50.0% similar within the C-domain to the IGFBP-1 sequences of chickens and humans, respectively. In contrast, catfish IGFBP-1 was 71.2% and 51.2% similar within the N-domain, 52.9% and 52.6% similar in the L-domain, and 79.8% and 65.4% similar within the C-domain to the IGFBP-1 sequences of zebra fish and rainbow trout, respectively (data not shown).

Weight gain was inhibited ($P < 0.001$) after 30 d of fasting. By day 30, Fed and Restricted fish weighed 107.5 ± 2.5 g and 67.1 ± 4.1 g, respectively. After 15 d of re-feeding, the Fed group weighed 137.5 ± 8.1 g, whereas the Restricted group weighed 86.3 ± 3.8 g.

Muscle and liver IGF-I mRNA decreased after 30 d of fasting (Fig. 2A and B, $P < 0.01$). After 15 d of re-feeding, IGF-I mRNA in the liver and muscle of the Restricted group was similar to that of the Fed control group. Liver IGF-II mRNA was not affected by fasting but was significantly greater in Restricted than in Fed fish after 15 d of re-feeding (Fig. 3A and B, $P < 0.05$). Abundance of muscle IGF-II mRNA was not affected by fasting and was similar to Fed control concentrations throughout the 45-d study. Concentrations of muscle and liver IGF-I mRNA were similar between treatments after re-feeding.

As expected, fasting increased liver IGFBP-1 mRNA ($P < 0.05$) and decreased IGFBP-3 mRNA (Fig. 4A and C, $P < 0.01$). Interestingly, re-feeding did not restore the concentrations of IGFBP-1 and IGFBP-3 mRNA to

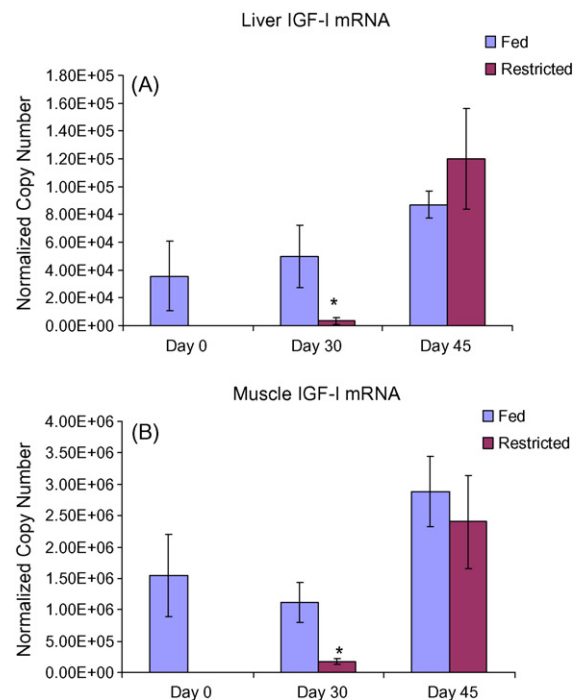


Fig. 2. Liver (A) and muscle (B) insulin-like growth factor-I mRNA concentrations in Fed control (commercial catfish diet fed daily); the other group of fish was subjected to a 30-day fast followed by 15 additional days of re-feeding (Restricted). N = 15 per treatment. Insulin-like growth factor-I copy number was normalized as a ratio to the amount of 18S. Standard error bars represent standard error of the mean, and significant differences ($P < 0.01$) are denoted by asterisks.

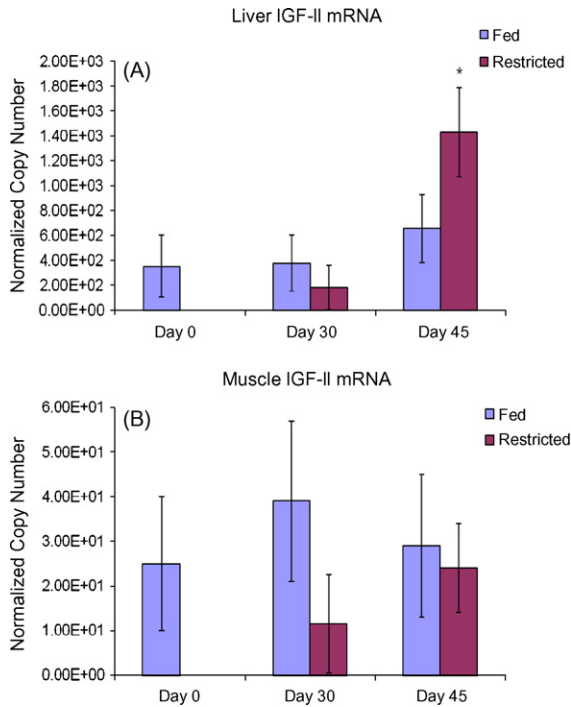


Fig. 3. Liver (A) and muscle (B) insulin-like growth factor-II mRNA concentrations in Fed control (commercial catfish diet fed daily); the other group of fish was subjected to a 30-day fast followed by 15 additional days of re-feeding (Restricted). N = 15 per treatment. Insulin-like growth factor-I copy number was normalized as a ratio to the amount of 18S. Standard error bars represent standard error of the mean, and significant differences ($P < 0.05$) are denoted by asterisks.

Fed control concentrations. Abundance of liver IGFBP-2 mRNA was not affected by fasting and was similar to Fed control concentrations throughout the 45-d study (Fig. 4B).

4. Discussion

A central component of growth coordination in mammals is the GH-IGF axis. The GH-IGF axis begins with the production of GH in the pituitary gland under the control of multiple hypothalamic hormones, including growth hormone releasing hormone (GHRH) and somatostatins (SS). Growth hormone circulates through the blood bound to growth hormone binding proteins, binds to its receptors, and stimulates IGF-I and IGF-II synthesis and secretion from the liver and other sites [38]. The IGFs evoke biological responses through their respective receptors, whose regulation is poorly understood. Insulin-like growth factor-I mediates many of the growth-promoting actions of GH [39], and it is becoming clear that the somatotrophic axis controlling vertebrate growth is highly conserved in fish [1,3].

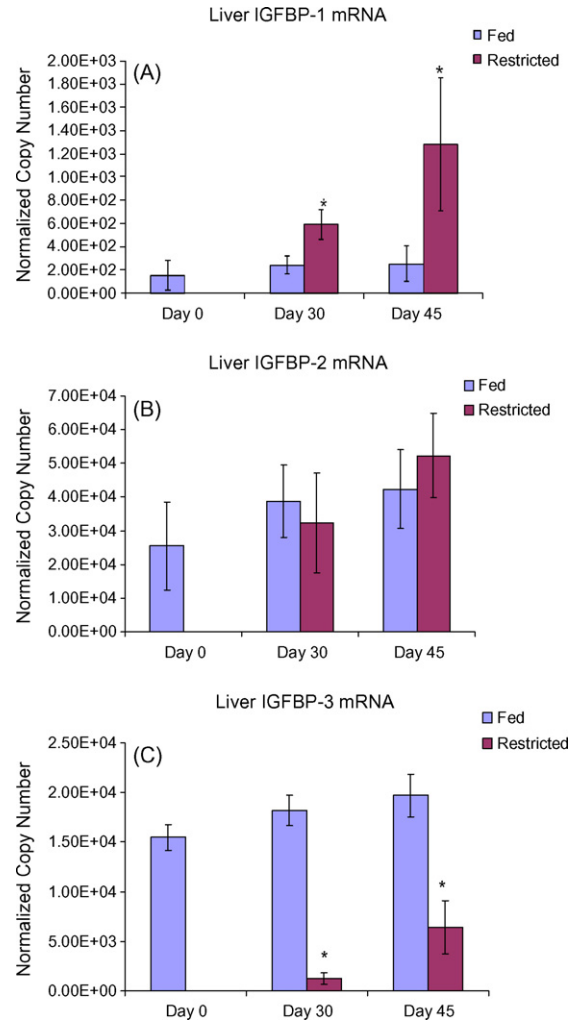


Fig. 4. Liver (A) insulin-like growth factor binding protein (IGFBP)-1, (B) IGFBP-2, and (C) IGFBP-3 mRNA concentrations in Fed control (commercial catfish diet fed daily); the other group of fish was subjected to a 30-day fast followed by 15 additional days of re-feeding (Restricted). N = 15 per treatment. IGFBP-1, IGFBP-2, and IGFBP-3 copy number were normalized as a ratio to the amount of 18S. Standard error bars represent standard error of the mean, and significant differences for IGFBP-1 ($P < 0.01$) and IGFBP-3 ($P < 0.01$) are denoted by asterisks.

Insulin-like growth factor-I plays similar roles in mammals and fish. In both species, IGF-I is regulated by nutritional status and plays a significant role in growth and development. In mammals, IGF-II mRNA is detected in fetal tissues but decreases quickly during postnatal development [39]. In contrast, teleostean tissues express substantial amounts of IGF-II mRNA later in life [40–42]. Growth hormone-dependent expression of IGF-II mRNA has also been demonstrated in fish [7,8].

Nutritional status regulated the IGF-IGFBP-growth axis in the present study. Fasting for 30 days reduced

fish weight by approximately 60% and caused significant changes in concentrations of IGF-I, IGFBP-1, and IGFBP-3 mRNA. Liver IGF-I mRNA returned to fed concentrations after 2 wks of re-feeding. Fasting has been shown to reduce plasma IGF-I and liver IGF-I mRNA concentrations in several species of fish [27,28,31], including channel catfish [33]. Muscle IGF-I mRNA also decreased after fasting and returned to fed concentrations after re-feeding. Since we did not measure muscle growth, it is too premature to suggest that IGF-I may play a role in the autocrine/paracrine regulation of muscle growth. Similar observations have also been found in rainbow trout [43]. It is also possible that nutritional status may have regulated the expression of muscle IGFBP(s), which could modulate the IGF-I autocrine/paracrine actions that have been observed in mammals [10]. The relative importance of liver-derived circulating IGF-I versus locally produced IGF-I is not clear, but it has been shown in mice that autocrine/paracrine IGF-I can support normal postnatal growth and development [44,45].

There is evidence that IGF-II is also related to local paracrine/autocrine regulation of tissue growth in teleosts [46]. We found that IGF-II mRNA concentrations in the muscle and liver were not affected by fasting. However, re-feeding caused IGF-II mRNA concentrations in the liver to increase 2.2-fold, whereas muscle IGF-II mRNA concentrations remained similar to fed fish. Liver IGF-II mRNA concentrations have been shown to be inversely proportional to rearing temperature in rainbow trout, whereas muscle IGF-II mRNA has been shown to gradually increase after re-feeding rainbow trout [43]. The role of IGF-II in growth in fish is not known, but it has been shown that handling and confinement stress result in a decrease in plasma concentrations of IGF-II in Atlantic salmon and rainbow trout [34], whereas starvation causes a 61% reduction in circulating concentrations of IGF-II in rainbow trout [35]. Peterson et al. [36,37] have shown that IGF-II mRNA is greater in faster-growing families of catfish, whereas Codina et al. [47] recently demonstrated that IGF-II has both mitogenic and metabolic effects in rainbow trout myocytes. In addition, Hevroy et al. [48] showed that muscle IGF-II mRNA was up-regulated 7-fold in salmon fed high lysine versus low lysine. The IGF results in catfish suggest that IGF-I and IGF-II are differently regulated by nutritional status and probably have a differential effect in promoting muscle growth during recovery from fasting. The IGFBPs are evolutionarily conserved components of the IGF system. Six forms of IGFBPs (1–6) bind IGF-I and -II with high affinity and regulate the IGFs available to their receptors, providing addi-

tional flexibility in regulating IGF signaling. Although it is not known whether these IGFBPs function similarly in teleosts as they do in mammals, evidence is growing that suggests IGFBPs may in fact play similar roles.

Kelley et al. [49] compared the IGF/IGFBP system in teleost and mammalian species and found that concentrations of IGFBPs in teleosts are also appreciably affected in anabolic and catabolic/stressful conditions such as fasting [50,38,51]. Insulin-like growth factor binding protein-1 in animals can inhibit IGF actions in vitro and in vivo [51]. Based on comparable molecular mass and an assumption of endocrine regulation similar to that in mammals, the ≤ 31 -kDa fish IGFBPs are proposed to be counterparts of mammalian IGFBP-1 or -2; and the 40- to 50-kDa fish IGFBP may correspond with mammalian IGFBP-3. The 40- to 50-kDa fish IGFBP demonstrates positive regulation by GH and is correlated with somatic growth in the striped bass (*Morone saxatilis*) [16,17], coho salmon (*Oncorhynchus kisutch*) [18,52], and tilapia (*Oreochromis mossambicus*) [19]. However, Peterson et al. [53] found that GH administration did not increase concentrations of a 45-kDa IGFBP in channel catfish.

In contrast, ≤ 31 -kDa fish IGFBPs are up-regulated in catabolic states and inversely correlated with somatic growth [54–56]. Davis and Peterson [54] showed that concentrations of a 24- and a 28-kDa IGFBP were increased after a 15-min, low-water stress in sunshine bass (*Morone chrysops* \times *saxatilis*). Siharath et al. [17] and Peterson et al. [24] demonstrated that fasting increased concentrations of a 25-kDa IGFBP in striped bass and a 20-kDa IGFBP in channel catfish. Similarly, in the longjaw mudsucker goby (*Gillichthys mirabilis*), fasting increased concentrations of the 24- and 30-kDa IGFBPs [56]. In addition, Kelley et al. [56] and Peterson et al. [24] observed increases in concentrations of cortisol, which is a principal hormone of catabolism, after fasting. This is an interesting finding, because IGFBP-1 gene expression in mammals is stimulated by glucocorticoids, resulting in elevations in serum IGFBP-1 concentrations [50]. Insulin-like growth factor binding protein-1 may play a role in sequestering available IGF peptide through high IGF binding affinity [50] and curtailing expensive anabolic functions during times of stress or nutritional deficiencies [56]. Kelley et al. [56] proposed that the measurement of lower-molecular-mass IGFBPs (≤ 31 -kDa fish IGFBP) might provide an assessment of growth status of fishes. In the present study, we report on the first real-time PCR assay for IGFBP-1, -2, and -3 in channel fish. We found that IGFBP-1 mRNA increased after fasting and continued to

be up-regulated even after 15 d of re-feeding. In previous studies, we showed that a 20 kDa IGFBP (catfish IGFBP-1) was increased after fasting [24] as well as in catfish fed dietary cortisol [25]. These 3 studies provide evidence that catfish IGFBP-1 is highly inducible under catabolic conditions such as food deprivation or when catfish are fed a cortisol-laden diet, which reduced growth and elevated plasma cortisol. Similar to our findings, Siharath et al. [16] reported an increase in concentrations of a 25-kDa IGFBP in fasting striped bass, whereas Kelley et al. [56] reported a 5-fold increase in concentrations of a 30- and 24-kDa IGFBP in fasted gobies. In zebra fish (*Danio rerio*), a 31-kDa IGFBP was also up-regulated with prolonged fasting [21].

The observed greater concentrations of IGFBP-1 mRNA after re-feeding suggest the catfish had not fully recovered from food deprivation. In vivo studies have indicated that the induced lower-molecular-weight IGFBPs may serve as a molecular switch to restricting IGF signaling and divert limited energy resources away from growth and development toward those metabolic processes essential for survival [57]. Our studies and others suggest that these lower-molecular-mass IGFBPs may be of general occurrence in teleosts that are metabolically regulated.

Evidence in zebra fish suggests IGFBP-2 is also a growth-inhibitory protein [21,58], whereas in the gilthead sea bream (*Sparus aurata*), IGFBP-2 may play a role in fish development and reproduction, particularly during gonadal development [22]. Fasting increases IGFBP-2 mRNA, whereas GH treatment decreases IGFBP-2 mRNA in zebra fish [21]. In channel catfish, there was no evidence to suggest that IGFBP-2 functions as a growth-inhibitory protein, as liver IGFBP-2 mRNA concentrations were similar in fed, fasted, and re-fed catfish. In addition, IGFBP-2 mRNA has been shown to be expressed in the adult skin, immature gonad, pyloric caeca, and kidney of the gilthead sea bream [22]. We did not examine IGFBP-2 mRNA concentrations in non-hepatic tissues, and it is possible that IGFBP-2 mRNA concentrations may have been induced in tissues such as muscle.

Insulin-like growth factor binding protein-3, which is regulated by GH and associated with anabolic growth in the striped bass [17], coho salmon [18], and tilapia [19], was significantly reduced in fasting catfish in the present study. In a previous study, we could not detect a significant increase in a 45 kDa IGFBP (catfish IGFBP-3) in the plasma of GH-injected catfish [53]. The lack of sensitivity of the Western ligand blot may have attributed to us not detecting differences in the plasma of GH-injected catfish. In the current study, we found that liver

IGFBP-3 mRNA concentrations remained low after re-feeding. This finding is similar to the results we found with IGFBP-1 mRNA; re-feeding for 2 wks did not cause IGFBP-3 mRNA concentrations to recover to fed control concentrations. The IGFBP-1 and IGFBP-3 mRNA results suggest that these 2 IGFBPs are metabolically regulated and IGFBP-1 is inversely correlated to somatic growth, whereas IGFBP-3 is positively correlated to somatic growth.

Sequence alignment and similarity to orthologous proteins in other vertebrates provided structural evidence that the 3 catfish sequences identified in the present research were IGFBP-1, -2, and -3. The catfish IGFBPs contained features common to other vertebrate IGFBPs, such as 12 conserved cysteines in the N-domain (exon 1), a highly variable L-domain (exon 2), and 6 conserved cysteines in the C-domain (exons 3 and 4) [20–22,49,57]. Multiple sequence alignment of catfish IGFBPs with vertebrate orthologues demonstrated sequence similarity in the N- and C-terminal domains (data not shown). That fact that the rainbow trout and Atlantic salmon IGFBP-3 were more similar to IGFBP-2 than IGFBP-3 in other teleosts, chickens, and humans indicates either a mistaken annotation of the salmonid sequences or unique IGFBP evolution in the salmonid lineage. We could not determine whether the catfish IGFBP-2 sequence contained the Arg–Gly–Asp sequence present in the C-terminal domain of all mammalian, chicken, and teleost IGFBP-2 proteins due to truncation of the available sequence. The putative heparin binding motif (PKKXRP) located in the central (L-) domain of all known mammalian, chicken, and teleost IGFBP-2 proteins was similar but not perfectly conserved in catfish (PKIPRP). Funkenstein et al. [22] also reported a lack of conservation of the heparin binding site in the gilthead sea bream. It has been suggested that binding of heparin and certain glycosaminoglycans to the heparin-binding domain in IGFBPs changes the conformation of those IGFBPs, resulting in a lower affinity to IGF-I [59], which would enable IGF-I to be released from the IGFBP complex to its respective receptor with subsequent induction of IGF-I effects [22].

In summary, sequence alignment and similarity to orthologous proteins in other vertebrates provided structural evidence that the 3 catfish sequences identified in the present research were IGFBP-1, -2, and -3. Fasting resulted in a reduction in muscle and liver IGF-I mRNA, a reduction in IGFBP-3 mRNA, and an increase in IGFBP-1 mRNA. Muscle and liver IGF-I mRNA concentrations were restored after 15 d of re-feeding, whereas liver IGFBP-1 and IGFBP-3 mRNA concentrations were not. Abundance of muscle IGF-II mRNA

was greater in catfish that had been fasted and then re-fed. Liver-derived catfish IGFBP-2 does not appear to be regulated by nutritional state, whereas IGFB-1 and -3 appear to metabolically regulated. These results also provide additional evidence of the conserved nature of the IGF-IGFBP-growth axis in catfish.

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